PERSISTENCE OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS IN IMMUNE ANIMALS AND ITS RELATION TO IMMUNITY

By ERICH TRAUB, V.M.D.

(From the Department of Animal and Plant Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

PLATE 51

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In a previous paper[1] it was reported that virus could be demonstrated in the blood and urine of mice and guinea pigs infected with acute lymphocytic choriomeningitis. Virus was also detected in nasal washings from mice infected naturally or experimentally. From an epidemiological viewpoint it is important to know whether or not the virus disappears from the blood, the secretions, and the excretions of immune animals.

Methods

The blood, urine, and nasal secretions of immune animals were tested for virus by inoculation into guinea pigs, which are highly susceptible and in which the disease can be diagnosed with certainty. Every infection, even the mildest subclinical one, produces in these animals a very solid immunity which can be detected by an inoculation with highly virulent virus given 3 to 4 weeks after the first inoculation. All guinea pigs which presented only fever or slight symptoms following inoculation were so tested.

Blood was repeatedly taken from the immune mice at long intervals in order not to disturb seriously the hematopoietic function. The blood was obtained by cardiac puncture under deep ether anesthesia, using a 0.25 cc. or 1 cc. tuberculin syringe and a very fine needle. At each bleeding 0.2 cc. blood was drawn and inoculated immediately (not defibrinated) into the brain of an etherized guinea pig. The heart puncture in mice proved to be a dangerous operation, but it was the only reliable method of obtaining standard amounts of blood. From guinea pigs blood was also obtained by cardiac puncture.

Urine was collected from mice and guinea pigs in sterile Petri dishes by exerting slight pressure on the abdomen in the region of the urinary bladder. Urine

[1] Traub, E., *J. Exp. Med.*, 1936, **63**, 533.

obtained in this manner was always free from blood but contaminated with bacteria, which, however, never interfered with the outcome of the experiments. Each urine sample was injected subcutaneously into the planta of a guinea pig.

Nasal washings were taken on mice and guinea pigs by repeatedly immersing their nostrils, each time for 5 to 10 seconds, in physiological saline (0.5 to 1 cc. per mouse) contained in a sterile Petri dish. The animals were not anesthetized. The nasal washings appeared slightly turbid. The saliva was not eliminated with certainty by this procedure. The inoculations were made subcutaneously into the plantae of guinea pigs. Bacteria present in the nasal secretions proved to be harmless to the test animals.

Mice were tested for immunity by intracerebral inoculation, under ether anesthesia, with 0.04 cc. of an infectious 5 per cent suspension of mouse brain. A group of 5 to 8 normal control mice from a stock free from choriomeningitis was included in each test. All control mice died in typical convulsions 6 to 7 days after the inoculation, while the mice whose immunity was being tested showed no symptoms, as indicated in Tables I, II, and III. Immunity tests in guinea pigs were carried out by a subcutaneous inoculation (0.25 cc. into each planta) with a virulent 10 per cent suspension of guinea pig brain. At least two normal control animals were inoculated.

As a source of virus for neutralization tests a 10 per cent suspension in saline of the brain of a guinea pig which had been killed when moribund following subcutaneous inoculation with a very virulent strain of the virus was used. For the tests this suspension was centrifugalized and known dilutions of the supernatant were mixed with equal amounts of undiluted serum. After incubation for ½ hour at room temperature, 0.25 cc. of each mixture was inoculated subcutaneously into each planta of a guinea pig. A comparative test has shown that smaller amounts of antivirus can be detected by the subcutaneous inoculation of guinea pigs than by the intracerebral injection of mice. The former were therefore used in these tests.

Tests for Virus in Blood, Urine, and Nasal Washings from Immune White Mice

The first observations on the persistence of active virus in the blood of immune mice were made with groups of mice from the infected stock which were inoculated with infective mouse brain suspension by different routes (15 mice intranasally, intraperitoneally, intracutaneously, and intracerebrally, respectively). Ten intracerebrally injected mice died, while the other 50 mice showed no symptoms. These mice were tested for immunity by an intracerebral inoculation with 0.04 cc. of an infective 1 per cent mouse brain suspension 3 weeks after the first inoculation and none of them showed any symp-

toms. 3 weeks later serum was collected from them (about 0.2 cc. was obtained from each mouse), and the pooled serum was filtered through a Berkefeld N candle. The filtered serum produced the disease in a guinea pig (0.25 cc. inoculated intracerebrally). The virus in the serum was inactivated by heating at 56°C. for ½ hour and no antivirus was detected in the heated serum. In a preliminary experiment with immune guinea pig serum it had been found that such heating did not affect antivirus.

From thirteen other mice which were injected with virus by different routes and failed to show symptoms following the first inoculation and the intracerebral test inoculation, serum was collected 4 weeks after the latter test. The pooled serum was not filtered, and it produced the disease when injected intracerebrally into a guinea pig. The virus in this serum was also inactivated by heating and no antivirus was detected in the heated serum.

Experiments carried out to determine the duration of the infection in immune mice in relation to their immunity are recorded in Tables I, II, and III.

In the experiment given in Table I seven mice were used which were left over from different experiments and which had received their first inoculations on different days. Mice 1 to 5 were obtained from the infected colony. Mice 3 and 4 had probably undergone a previous natural infection, since neither showed symptoms following an intracerebral inoculation with 0.04 cc. of virulent 5 per cent mouse brain suspension. Mice 1 and 2, which showed typical symptoms following intracerebral inoculation with the same amount of virus and recovered, had probably not been infected prior to the inoculation. Mouse 5 may have been infected prior to the intranasal instillation of 0.05 cc. of the supernatant fluid of a 5 per cent mouse brain suspension, because intranasal instillations of virus in mice as a rule produce no symptoms. Mice 6 and 7 were descended from the infected stock, but were bred from disease-free parents and were not infected prior to the intranasal instillation of a 1 per cent guinea pig brain suspension carried out according to the method described previously.[1] The results of the blood tests are given in Table I.

Table II records tests for virus in seven mice which were inoculated intraperitoneally with 0.5 cc. of a 1 per cent guinea pig brain suspension, were very sick from the 7th to about the 14th day after the inoculation, and recovered. On the 27th day their immunity was tested by intracerebral inoculation with virus. The surviving mice were again inoculated intracerebrally 83 and 166 days, respectively, after the first test for immunity and none of them showed symptoms.

TABLE I

Tests for Virus in the Blood of Immunized Mice

Mouse No.....	1	2	3	4	5	6	7
					1st inoculation inas. No symptoms		
Time after 1st inoculation	1st inoculation ic followed by typical symptoms and recovery Test inoculation 50 days after 1st inoculation	Inoculated ic Typical symptoms, recovery No test inoculation	Inoculated ic. No symptoms No test inoculation	1st inoculation ic No symptoms Test inoculation ic 28 days after 1st inoculation	Test inoculation ic 28 days after 1st inoculation	Test inoculation ic 15 days after 1st inoculation	1st test inoculation ic 15 days after 1st inoculation 2nd test inoculation ic 143 days after 1st inoculation
	Results of blood inoculations (0.2 cc. heart blood of each mouse injected ic into a guinea pig)						
days							
Shortly before 1st inoculation	—	—	—	—	—	0	0
11	—	—	—	—	—	D 16	D 15
26	0	F, S, I	D 20	—	—	—	—
28	—	Died of injury	—	0*	0*	—	—
42	—	following	—	0	0	—	—
50	0*	bleeding on	D 16	Died of injury	Killed for se-	0	D 14
64	0	26th day	Died of injury	following	rum on 70th	—	D 25
110	Killed for se-		following	bleeding on	day	—	0
135	rum on 92nd		bleeding on	42nd day		0	0
143	day after 1st		50th day			0	0*
158	inoculation					Died of injury	F, S, I
178						following	0
						bleeding on	
						143rd day	

ic = intracerebrally; inas = intranasally. 0 = no fever, no symptoms, not immunized. D 16 = died in 16 days. — = no test made. F = fever. S = typical symptoms followed by recovery. I = immunized.

* The test inoculations were made immediately after the bleedings.

TABLE II

Tests for Virus in the Blood and Urine of Immunized Mice

Time after 1st test of immunity	Inoculations into guinea pigs of heart blood from							Inoculations into guinea pigs of pooled urine from mice 8–14	
	Mouse 8	Mouse 9	Mouse 10	Mouse 11	Mouse 12	Mouse 13	Mouse 14	Amount	Result
days								*cc.*	
30	+ F, S, I	0	0	+ F, S, I	+ D 27	0	+ D 21	0.7	+ F, S, I
65	Died of injury on	0	0	" " "	+ D 15	0	+ D 17	0.25	" " "
83*	30th day	0	0	" " "	+ D 17	0	Died of injury on	–	–
98		+ F, S, I	+ F, S, I	" " "	+ F, severe S, I	+ F, S, I	65th day	1.4	0
133		0	0	0	+ F, S, I	0		0.85	0
166*		0	0	–	–	–		–	–
175		Killed on 166th day. No virus detected in organs		Blood plasma + D17	Blood plasma + F, severe S	Blood plasma + D 17		–	–
				Washed blood cells 0	Washed blood cells 0	Washed blood cells 0			
207				0	+ F, S	0		–	–

+ = virus detected. 0 = no fever, no illness, and no immunity. D 27 = died in 27 days. – = no test made. F = fever. S = typical symptoms. I = immunized.

* The surviving mice were tested for immunity.

Blood and organs from mice 9 and 10, killed on the 166th day, were examined. No virus was detected in the brain and spinal cord, lungs, heart, liver, spleen, kidneys, urinary bladder, salivary glands, uteri, ovaries, or several lymph nodes including the mediastinal and mesenteric ones. These determinations were made by injecting the total amount of each suspension of an entire organ into a guinea pig (amounts of 2 cc. and less were injected subcutaneously into the plantae; if there was more suspension than 2 cc., the remainder was injected intraperitoneally). The suspensions of several small organs, for instance those of the salivary glands and submaxillary lymph nodes or of the ovaries and the uteri, were pooled and so injected.

On the 175th day the blood plasma and washed blood cells of mice 11, 12, and 13 were tested for virus. 0.2 cc. heparinized plasma from each mouse was injected intracerebrally into a guinea pig. The blood cells from each mouse were washed three times in 10 cc. Tyrode solution containing heparin. From the centrifuged washed blood cells of each mouse the upper layer containing the bulk of the white cells and many erythrocytes was drawn off with a capillary pipette, and enough Tyrode solution was added to make each inoculum 0.25 cc., which was injected intracerebrally into a guinea pig. Virus was detected in the heparinized plasma of all three mice, but not in the washed blood cells.

Table III records tests for virus made upon mouse 15 over a period of more than 7 months. This animal, which had undergone a natural infection, was about 7 weeks old when it was removed from the infected stock. It grew normally and appeared to be in perfect health throughout the experiment. Its immunity was confirmed by intracerebral inoculation 143 days after its removal from the colony. The regularity with which virus was detected in the urine and nasal washings is remarkable. The virus seems to have been continuously present in the urine, and very small amounts of urine produced the disease in guinea pigs.

On the 225th day mouse 15 was sacrificed for histological examination and virus was demonstrated in the blood plasma and washed blood cells as well as in the thoroughly washed spleen tissue. Other organs were not tested for virus.

In another experiment the nasal washings of eleven immune mice were tested for virus. These mice had been infected by intravenous injection with 0.25 cc. supernatant fluid of a virulent 10 per cent guinea pig brain suspension. Six mice were sick from the 6th to the 10th day after the inoculation and then recovered completely, while five showed no definite symptoms. Pooled nasal washings obtained on the 39th, the 56th, and the 121st day after inoculation produced

TABLE III

Tests for Virus in the Blood, Urine, and Nasal Washings from Mouse 15

Time after removal from infected stock	Blood	Urine		Nasal washings	
	Result (0.2 cc. injected ic into 1 guinea pig)	Amount	Result (each specimen injected sc into 1 guinea pig)	Amount	Result (each specimen injected sc into 1 guinea pig)
hrs.		*cc.*		*cc.*	
1	D 15				
days					
25	F, no S, I				
49	F, slight S, I				
64	D 17				
110	F, slight S, I	0.3	F, no S, I		
123		0.35	F, severe S, I		
126		0.2	F, severe S*		
127		0.1	" " "		
135		0.15	" " "	0.7	F, severe S*
143†	F, no S, I				
158	F, severe S*	0.02	D 12		
162				0.7	D 16
168		0.02	D 15	0.7	F, slight S, I
184		0.01	F, slight S, I	0.7	F, severe S*
192		0.15	F, severe S, I	0.8	F, severe S, I
198		0.05	F, no S, I	0.8	" " " "
213		0.4	F, severe S, I		
214		0.02	" " " "		
215		0.02	" " " "		
216		0.01	" " " "		
225	Blood plasma and washed blood cells F, severe S*				

D 15 = died in 15 days.

F = fever.

S = typical symptoms of choriomeningitis followed by recovery.

I = immunized.

* Since the guinea pig showed unmistakable symptoms, no test inoculation was made.

† Tested for immunity by intracerebral inoculation with virus immediately after bleeding.

the disease in guinea pigs. No attempt was made to determine how many mice had remained carriers of the virus.

The experiments just presented indicate that some mice remain carriers of the virus for several months after clinical recovery, while

others cannot be shown to carry it after a short time. The number of mice examined is too small to allow a conclusion on the percentage of immune carriers.

Tests for Virus in Blood, Urine, and Nasal Washings from Immune Guinea Pigs

Tests have been made with blood of guinea pigs (whole blood, washed corpuscles, and plasma were tested in two cases) drawn on

TABLE IV

Tests for Virus in the Urine of Immune Guinea Pigs

Time after test of immunity	Guinea pig 1		Guinea pig 2		Guinea pig 3		Guinea pig 4		Guinea pig 5	
	Character of disease									
	Very severe (31 days)*		Very severe (31 days)*		Fever, no symptoms (37 days)*		Fever, slight symptoms (30 days)*		Fever, no symptoms (30 days)*	
	Subcutaneous inoculations of urine into guinea pigs									
	Amount	Result	Amount	Result	Amount	Result	Amount	Result	Amount	Result
days	*cc.*		*cc.*		*cc.*		*cc.*		*cc.*	
18							0.5	0		
19	0.15	+ D 17	0.25	+ D 13	0.8	0			0.7	0
27			0.5	+ D 22						
35	0.5	0	0.2	0						

\+ = virus detected.

0 = no fever, no symptoms, and no immunity.

D 17 = died in 17 days.

* Number of days which elapsed between inoculation and test of immunity.

the 35th day after the immunizing inoculation or later, but we have thus far been unable to demonstrate virus in it, even when several cubic centimeters were injected into susceptible guinea pigs. In the sera of immune guinea pigs antivirus is readily demonstrable and is probably responsible for the inactivation of the virus in the blood.

Tests for virus in the urine of five immune guinea pigs are recorded in Table IV. These animals were infected by subcutaneous inoculation with virus and their immunity was tested in the same way. The blood of each guinea pig was tested for virus by inoculat-

ing 0.2 cc. intracerebrally into a guinea pig, with a negative result. The symptoms in the two guinea pigs whose urine contained virus had been severe, while in the others they were mild.

In another experiment virus was detected in pooled urine from five guinea pigs that had shown severe symptoms following subcutaneous inoculation with virus 35, 36, 37, 40, or 44 days previously. The animals received no tests for immunity. Shortly after the collection of the urine each guinea pig was bled, and its defibrinated blood was injected into a guinea pig (0.2 cc. intracerebrally, 3 cc. intraperitoneally), with negative result. Pooled nasal washings taken from these five guinea pigs were avirulent. No other tests have been made with nasal washings from immune guinea pigs.

Virus was also present on the 23rd day after inoculation in the urine of a guinea pig which had recovered from choriomeningitis of moderate severity. The blood serum of this guinea pig was avirulent and contained some antivirus at that time (test recorded in Table V).

Experiments Bearing on the Mechanism of the Immunity to Choriomeningitis

The immunity produced in mice by inoculation with virus arises rapidly. When mice injected intraperitoneally with virus are given an intracerebral test inoculation on the following day, they all die in typical convulsions. When, however, the test inoculation is given 5 days after the first injection, some mice will be immune. Virus injected intracerebrally on the 8th day has no effect, even though some mice still show symptoms of the disease. The occasional persistence of virus in the blood of mice after recovery suggested an investigation into the relation between the presence of the virus and immunity.

Mice in whose blood no virus had been demonstrated and others whose blood was virulent were tested for immunity by intracerebral injection with virus. Records on such mice are given in Tables I, II, and III (mice 1, 4, 5, 7, 9, 10, 11, 12, 13, and 15). It is evident that mice which carried virus in their blood were just as resistant to reinoculation as those which did not. Numerous other tests carried out with mice from the infected stock confirmed this observation.

It was of interest to determine whether circulating antivirus ac-

counted for the apparent disappearance of the virus from the blood of some immune mice. Neutralization tests were therefore carried out with three sera obtained from immune mice in whose blood no virus was detected in repeated tests.

Immune Serum I.—Pooled sera from mice 1 and 5 (Table I). Subcutaneous injection of 0.25 cc. of this serum into a guinea pig produced no disease.

Immune Serum II.—Pooled sera from mice 9 and 10 (Table II) drawn on the 166th day, and from mouse 7 (Table I) drawn on the 227th day. Intracerebral inoculation of 0.3 cc. of this serum into a guinea pig was without effect.

Immune Serum III.—Pooled sera from five immune, full grown mice from the infected stock. A guinea pig inoculated intracerebrally with 0.3 cc. of this serum showed no reaction.

TABLE V

Neutralization Tests with Three Sera from Immune Mice and the Serum of a Convalescent Guinea Pig Whose Urine Contained Virus

Virus dilution	Results of inoculations of the serum-virus mixtures subcutaneously into guinea pigs (1 guinea pig used for each mixture)						
	Experiment 1			Experiment 2			
	Immune mouse serum I	Normal mouse serum (control)	Normal horse serum (control)	Immune mouse serum II	Immune mouse serum III	Normal mouse serum (control)	Convalescent guinea pig serum
10^{-1}	+	+	−	−	−	−	−
10^{-2}	+	+	+	+	+	+	+*
10^{-3}	+	+	+	+	+	+	+*
10^{-4}	0	+*	0	+	+	+	0
10^{-5}	−	0	0	0	0	0	−

\+ = died of choriomeningitis or was killed when very sick.
0 = showed no fever or symptoms.
− = not tested.
* Delayed disease.

The tests presented in Table V show that all three of these sera had practically no neutralizing power under the conditions of the experiment.

In contrast to the results in mice, numerous tests with sera from immune guinea pigs have invariably shown antivirus to be present.

Histological Examination of Tissues from Immune Animals Which Carried Virus

Material from seven naturally infected mice was examined histologically. These mice had shown no clinical evidence of disease while under observation and carried virus in their blood for several months. Virus was demonstrated in the blood of each mouse shortly before it was chloroformed.

Mouse 15 (Table III), killed on the 225th day after its removal from the infected stock, showed at autopsy a somewhat enlarged spleen and a nutmeg colored liver. No other changes were noted. The lungs appeared normal. The brain, spinal cord, lungs, heart, liver, spleen, kidneys, urinary bladder, as well as the femoral and vertebral bone marrow, were examined histologically. The lungs showed areas of marked interstitial pneumonia. There was an interstitial hepatitis and large round cell collections in the neighborhood of blood vessels (Fig. 1). Smaller collections of lymphocytes and some polymorphonuclear leucocytes were scattered over the section and gave it a spotted appearance. Kidney sections showed small areas of interstitial nephritis. In the spleen the Malpighian bodies were enlarged, and the number of megakaryocytes was increased. In the rest of the material studied no definite changes were noted.

Mouse A carried virus in the blood for at least 3 months. At autopsy no definite changes were seen. The liver, spleen, and kidneys were examined histologically. In the liver there was a patchy hyperplasia of Kupffer cells. The spleen showed no lesions, but sections through the kidneys presented small areas of interstitial nephritis.

Mouse B carried virus in the blood for at least 5 months. No changes were found at autopsy. Histologically a reticuloendothelial hyperplasia was detected in the liver, and kidney sections showed a patchy interstitial nephritis. The spleen appeared normal. No other organs were examined.

Mouse C carried virus in the blood for at least 4 months. At autopsy no definite changes were noted. The lung section showed areas of interstitial pneumonia. In the liver and spleen no definite changes were present. The kidneys showed a slight interstitial nephritis.

Mouse D carried virus in the blood for at least 6 months. Its respiratory rate was increased. At autopsy a mediastinal tumor was noted which filled about one-half of the thoracic cavity. The spleen was enlarged (about two to three times normal volume). Histologically the tumor appeared as a lymphosarcomatous mass containing an extremely large number of cells in mitosis. The lungs presented an interstitial pneumonia with round cell collections in the neighborhood of blood vessels and bronchi and in the lung tissue. The liver showed small round cell collections around blood vessels and bile ducts. In the spleen the Malpighian

bodies were enlarged, and the red pulp was infiltrated with lymphocytes. Kidney sections showed a slight interstitial nephritis.

Mouse E carried virus in the blood for at least 6 months. At autopsy no gross lesions were noted. Histologically an interstitial pneumonia (Fig. 2) and a marked interstitial nephritis (Fig. 3) were detected. The liver, spleen, and mediastinal lymph nodes showed no definite changes.

Mouse F carried virus in the blood for at least 6 months. At autopsy no gross lesions were present. A slight interstitial pneumonia and a marked interstitial nephritis were noted in sections. The liver tissue contained small collections of round cells. The spleen and mediastinal lymph nodes appeared normal.

In the mice examined lesions were most frequently found in the lungs and kidneys. While it cannot be proved that these lesions are caused by choriomeningitis virus, it must be noted that they resemble the more marked lesions which are present in mice acutely ill following intravenous injection with the virus.

Sections through the lungs, liver, spleen, and kidneys of two full grown mice from the infected stock which had themselves been infected with choriomeningitis, but in whose blood no virus was detectable, showed no changes. In four other mice slight changes of the same character as those described above, but less marked, were noted. Traces of an interstitial pneumonia and small round cell collections near blood vessels were present in one mouse. In three cases small interstitial round cell collections were seen in liver sections. They were much less extensive than those in mice carrying virus. In one mouse a marked, patchy interstitial nephritis was noted, while in three cases only traces of interstitial nephritis were found.

Tissues from seven normal mice were examined histologically. These mice were bred from disease-free parents, and tests made shortly before the tissues were removed disclosed no pathogenic virus in the blood. The lungs and kidneys showed no lesions. In the lungs there were no peribronchial, perivascular, or interstitial round cell collections, such as are believed by some investigators to be present under normal conditions. Liver sections showed no changes in five cases, while in two cases a few small collections of polymorphonuclear leucocytes and round cells were present in the interstitial tissue. The collections consisted of less than ten cells each and were much less numerous and extensive than those noted in mice carrying the virus. There was no hyperplasia of Kupffer cells.

A guinea pig which had recovered from choriomeningitis of moderate severity was sacrificed on the 28th day after the subcutaneous inoculation with virus. It discharged virus with the urine at that time, but no virus was detected in the blood. Kidney sections revealed large perivascular round cell infiltrations and a marked interstitial nephritis very similar to that noted in mice.

Dr. T. F. McNair Scott of New York has kindly permitted me to refer here to his recent experiments in which he detected both lesions and virus in the central nervous systems of mice which had recovered from choriomeningitis following intracerebral inoculation with virus administered 14 or 28 days previously.

DISCUSSION

From the histological findings just outlined it seems possible that in animals which have recovered clinically from choriomeningitis but continue to carry active virus and to discharge it, the virus persists in lesions in the lungs, kidneys, and liver. From these lesions it may get into the circulation, and from the blood into the urine, or directly into the urine if lesions are in the urinary system. The lung lesions in mouse 15 were perhaps responsible for the presence of virus in the nasal secretions. The virus seemed to have multiplied continuously in this animal, because it discharged considerable amounts of virus over a long period of time. The presence of slight lesions in mice which once were infected, but in whose blood no virus was detected, may be taken to indicate that the discharging of virus into the blood ceases before the lesions have completely disappeared. In guinea pigs immune to choriomeningitis, in whose blood antivirus is present but which discharge virus with their urine, the virus may be situated intracellularly in kidney lesions and thus protected from the action of circulating antivirus.

In mice 6, 7, and 11, as well as in several other naturally infected mice not dealt with here, the virus gradually disappeared from the blood in the course of time. Neutralization tests indicated that circulating antivirus was not responsible for this gradual elimination, which perhaps ran parallel with the healing of the lesions.

When immune mice whose blood has been avirulent on repeated tests are reinoculated, the virus may circulate for some time (mice 7, 9, 10, 11, and 13; in mice 1, 4, and 5 the virus probably also circulated for some time following the test inoculation but was eliminated more rapidly than in the former mice). It is unlikely that new lesions are produced by the reinoculations. The time of circulation of the virus is comparable with that of antivirus in passively immun-

ized animals. In guinea pigs passively immunized to pseudorabies this time was found to vary between 1 and 4 weeks (unpublished experiments).

The immunity to choriomeningitis in mice does not seem to be dependent upon the presence of active virus in the body (mice 9 and 10). Unfortunately it cannot be definitely proved with the methods available at present that the whole body of the animal is free from virus, since subinfective amounts of virus may not be recognized. There is, however, reason to believe that the process of gradual elimination of the virus mentioned above continues to completion. Most of the evidence obtained with other viruses is in favor of this assumption. The evidence for the contrary view is not convincing.

While antivirus may be present in too low a concentration to be detected by the method employed, the failure to demonstrate it in sera from solidly immune mice is evidence that it does not determine the immunity of mice to choriomeningitis. The continued presence of virus in the blood plasma of immune mice (mice 11, 12, and 13) suggests that phagocytes also play no part in the immunity. It is tentatively concluded that tissue immunity is the essential factor in mice. In immune guinea pigs whose sera invariably possess neutralizing properties, the antivirus may be an essential immunity factor.

SUMMARY

In some apparently healthy mice the virus of lymphocytic choriomeningitis persisted for a considerable period of time after recovery, in the blood, urine, and nasal secretions, while in other mice it soon became undemonstrable. It is possible that the persistence of the virus is due to lesions in the lungs, liver, and kidneys.

The immunity to lymphocytic choriomeningitis in mice does not seem to depend upon the presence of virus in the blood and the organs tested. No antivirus was detected in sera from several solidly immune mice, which fact suggests that circulating antivirus plays no important part in their immunity. Leucocytes also seem to be no essential factor in this immunity, which probably is closely linked with the tissues.

The urine of guinea pigs which had recovered from severe attacks

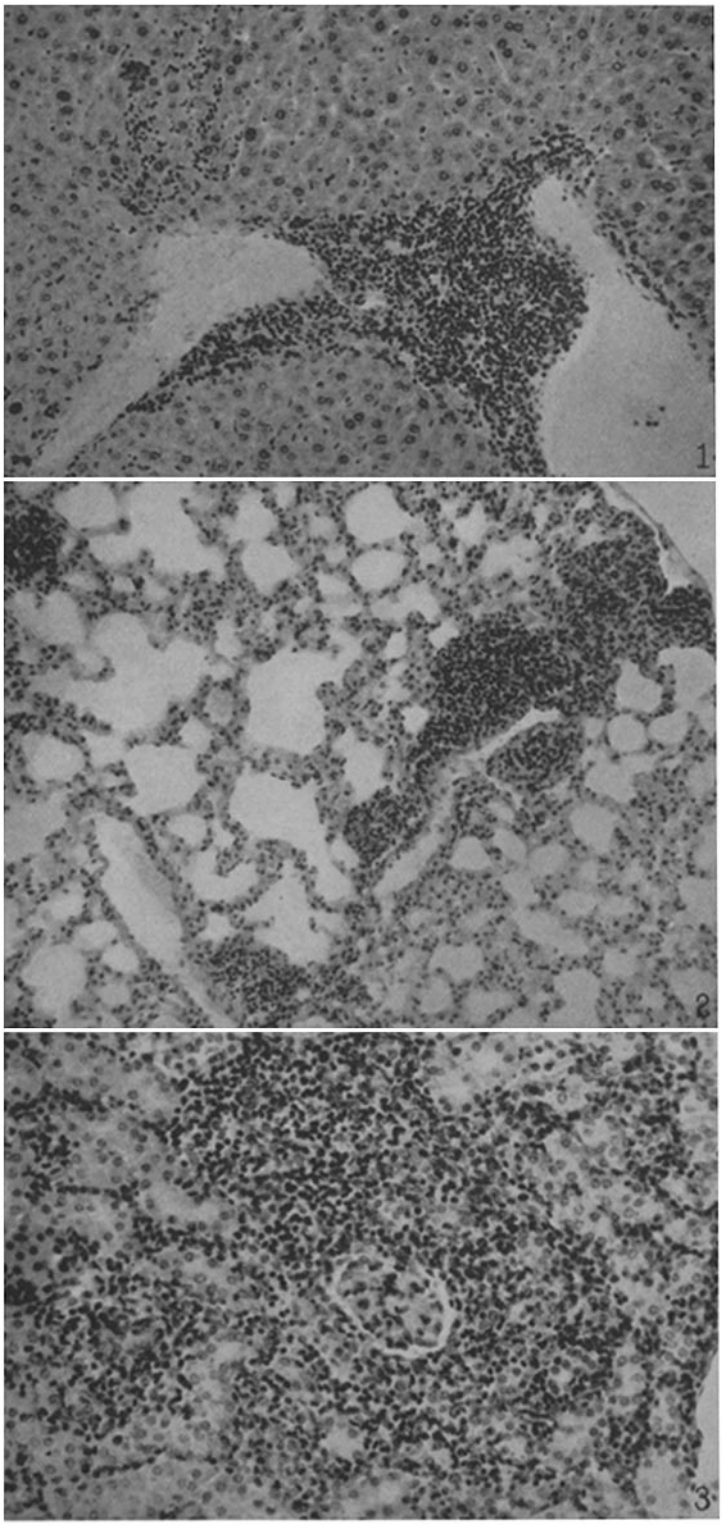
of lymphocytic choriomeningitis contained virus for a few weeks after recovery, while that from mild cases contained no virus. Virus was never demonstrated in the blood of immune guinea pigs. Antivirus was readily detected in it.

EXPLANATION OF PLATE 51

FIG. 1. Section through liver of mouse 15. Large round cell collection. Note area of interstitial hepatitis. Hematoxylin and eosin. × 220.

FIG. 2. Section through the lung of immune mouse E showing area of slight interstitial pneumonia with round cell collections. Hematoxylin and eosin. × 220.

FIG. 3. Section through kidney of immune mouse E showing an area of marked interstitial nephritis. Hematoxylin and eosin. × 332.



Photographed by J. A. Carlile

(Traub: Choriomeningitis virus in immune animals)